

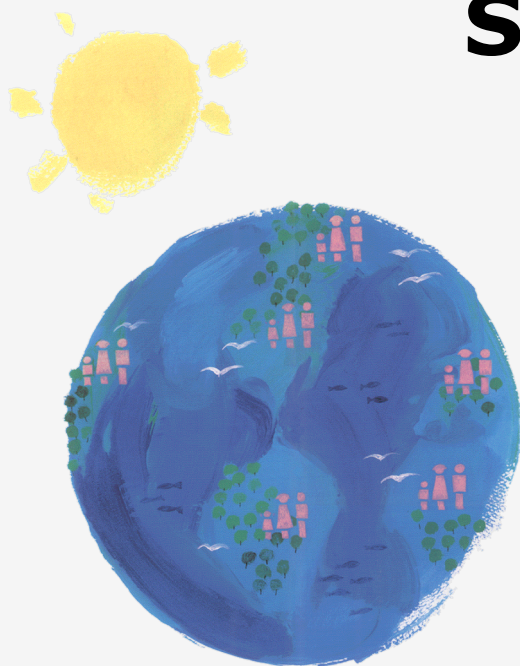
NICEATM

*National Toxicology Program Interagency
Center for the Evaluation of Alternative
Toxicological Methods*

ICCVAM

*Interagency Coordinating Committee on
the Validation of Alternative Methods*

Nominations and Submissions to ICCVAM



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SACATM Meeting
December 12, 2005
Alexandria, Virginia



Nominations and Submissions

- The Humane Society of the United States Nomination: Alternative Methods to Replace the Mouse LD₅₀ Assay for Botulinum Toxin Potency Testing
- The European Centre for the Validation of Alternative Methods (ECVAM) Submission: 5 *In Vitro* Pyrogenicity Test Methods



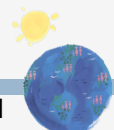
Nomination by the Humane Society of the United States

Alternative Methods to Replace the Mouse LD₅₀ Assay for Botulinum Toxin Potency Testing



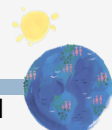
Botulinum Toxin Potency Testing Timeline

- 31 Oct 05 HSUS nomination received.
- 16 Nov 05 ICCVAM recommends accepting the HSUS nomination and establishes the ICCVAM Biologics Working Group (BWG).
- 27 Nov 05 Initial contact with ECVAM to identify a liaison for the BWG and to coordinate efforts.
- 12 Dec 05 SACATM considers the HSUS nomination.



HSUS Test Method Nomination

- The HSUS nomination “*Alternative Methods to Replace the Mouse LD₅₀ Assay for Botulinum Toxin Potency Testing*” included:
 - A brief review of existing *in vivo*, *ex vivo*, and *in vitro* methods as potential alternatives to the mouse *Clostridium botulinum* neurotoxin (BoNT) potency assay.
 - A recommendation to hold an expert workshop to expeditiously review existing methods.
 - A recommendation that, after the workshop, ICCVAM work with appropriate partners to validate one or more of these alternative assays.



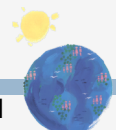
Applicability to Regulatory Testing Needs and to Multiple Agencies or Programs

- Applicability to Regulatory Testing Needs: Botox, Botox Cosmetic, and MyoBloc are regulated as drugs by the FDA.
- Multiple Agency Applicability: the following agencies require, request, or use the mouse LD₅₀ assay for BoNT potency, diagnostic, or detection testing - FDA, CDC, USDA, DOD, EPA.
- Warranted: the increasing use of these drugs requires increased numbers of animals used for potency testing.



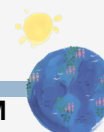
The Potential for the Proposed Test Method to Refine, Reduce, or Replace Animal Use

- Each batch of BoNT may be tested up to 3 times prior to use.
- Each potency test uses 100 or more mice.
- Mice are injected intraperitoneally, and monitored for up to 4 days for death to determine the dose that kills 50% of the animals.
- Death results from suffocation due to paralysis.
- Alternatives would reduce, refine, and replace animal use.



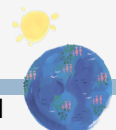
The Potential for the Proposed Test Method to Improve Prediction of Adverse Health Effects

- Variability in the assessment of potency has been cited as one problem of the mouse bioassay; a standardized, cell-based assay or analytical assay may have greater reliability
- The mouse assay is relatively slow and a validated alternative assay could be potentially much faster, thereby providing improved and quicker prediction of adverse health and environmental effects.



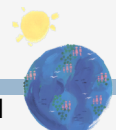
The Extent to Which the Test Method Provides Advantages Compared to Current Methods

- As stated, alternatives could potentially be more reliable, faster, and less costly than the current mouse potency assay.



Completeness of the HSUS Nomination with Regard to ICCVAM Submission Guidelines

- HSUS states that the nomination packet represents the current status of the available methods and the nomination is for holding an ICCVAM Workshop to expeditiously review the validation status of existing alternative test methods.



Future BoNT Potency Testing Activities

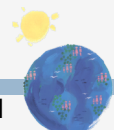
Dec 2005 Publish a *Federal Register* notice requesting

- public comment on the nomination
- identification of other test methods that might be considered
- alternative test data for consideration
- candidates for a possible expert workshop

Jan 2006 ICCVAM Meeting: Recommendations finalized and if a workshop recommended with a high priority, start process to:

- identify participants for Expert Workshop
- develop supplemental information on alternative assays and questions for the Expert Workshop Panel

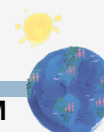
2006 Possible ICCVAM/ECVAM workshop to review alternative test methods and establish priorities for pre-validation/ validation studies



ECVAM Submission

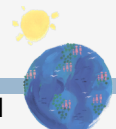
5 *In Vitro* Pyrogenicity Test Methods

- PBMC/IL-6 (The Human PBMC/IL-6 *In Vitro* Pyrogen Test)
- WB/IL-1 (The Human Whole Blood/IL-1 *In Vitro* Pyrogen Test)
- cryo WB/IL-1 (The Human Whole Blood/IL-1 *In Vitro* Pyrogen Test: Application of cryopreserved human whole blood)
- WB/IL-6 (The Human Whole Blood/IL-6 *In Vitro* Pyrogen Test)
- MM6/IL6 (An Alternative *In Vitro* Pyrogen Test Using the Human Monocytoid Cell Line MONO MAC-6 [MM6])



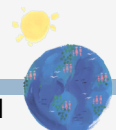
Pyrogenicity Test Methods Timeline

- 24 Jun 05** ECVAM submits five *in vitro* pyrogenicity test methods to ICCVAM for review
- 25 Jul 05** Request to ICCVAM-member Agencies for nominations of Pyrogenicity Working Group (PWG) members
- Sep 05** The ICCVAM PWG established
- 04 Oct 05** NICEATM pre-evaluation and ECVAM submitted information sent to the PWG for review and comment
- 25 Oct 05** Inaugural PWG meeting to draft recommendations for priority and future review activities
- 16 Nov 05** ICCVAM considers the PWG draft recommendations
- 12 Dec 05** SACATM meeting - considers ECVAM submission



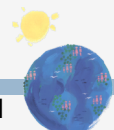
ECVAM Test Method Submission

- The five assays involve the measurement of cytokine levels (either IL-1b or IL-6) from human whole blood, peripheral blood mononuclear cells (PBMC), or a human monocytoïd cell line treated with a sample of interest.
- Cytokines are used as biomarkers of a pyrogenic response
- In each assay, cytokine levels are measured with an enzyme-linked immunosorbent assay (ELISA).



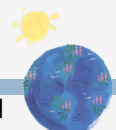
NICEATM Prescreen Evaluation

- The five individual BRDs were reviewed for completeness and to identify aspects or omissions that could impede an expert peer review.
- The BRDs were not reviewed with respect to data quality or validation study conclusions.
- The adequacy of each submission was evaluated based on the following criteria:
 - The extent to which the submissions provide the information requested in the ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods (NIH Pub. No. 03-4508).
 - The extent to which the submissions address the ICCVAM prioritization criteria.



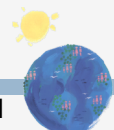
Applicability to Regulatory Testing Needs

- There are regulatory requirements to test pharmaceutical products (human and veterinarian drugs, devices) for pyrogenicity. The pyrogenicity assays that are currently acceptable to regulatory authorities require intact animals (rabbits) or an *in vitro* test that requires the use of amebocyte lysate extracted from *Limulus polyphemus* (bacterial endotoxin test; BET).
- According to the BRDs, “dependent on the product and the presence of relevant clinical data on unexpected pyrogenicity of clinical lots, the proposed test method[s] may be an alternative method for pyrogen testing, thus substituting [for] the rabbit pyrogen test or the BET. In certain cases, the proposed test method may function as a supplementary test method to assess compliance to the licensing dossier.”



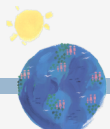
Applicability to Multiple Regulatory Agencies or Programs

- These methods will reportedly be applicable to all agencies and programs that require pyrogenicity testing of pharmaceuticals and other products. FDA requires that human injectable drugs (including biological products), animal injectable drugs, and medical devices be tested for the presence of pyrogenic substances.
- The relevant FDA Centers are:
 - Center for Biologics Evaluation and Research (CBER)
 - Center for Drug Evaluation and Research (CDER)
 - Center for Devices and Radiological Health (CDRH)
 - Center for Veterinary Medicine (CVM)
- The relevant European Union (EU) Authorities are:
 - European Medicines Agency (EMA)
 - Regulatory Authorities for individual EU countries
 - European Pharmacopoeia Commission



Extent of Expected Use or Application and Impact on Human Health

- Under certain circumstances, the proposed tests are intended to replace tests that are currently used widely in pharmaceutical development (i.e., *in vivo* rabbit pyrogen test, BET).
- These proposed tests are allegedly as good as, if not better than, current test methods for identifying both endotoxin and non-endotoxin pyrogens.
 - They may offer improved prediction of a pyrogenic response from products intended for humans, and subsequently provide greater protection of human health.



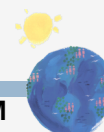
The Potential for the Proposed Test Methods to Refine, Reduce, or Replace Animal Use

- The two most common pyrogen tests presently used (i.e., *in vivo* rabbit pyrogen test, BET) require the use of animals
 - The rabbit pyrogen test requires ≥ 3 rabbits/test
 - The BET is performed using blood drawn from *L. polyphemus* which may be returned to the wild; however, some of these animals do not survive the procedure
- The proposed test methods rely on human blood cells or a human monocytoid cell line that can be cultured in the test laboratory.



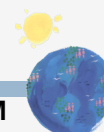
The Potential for the Proposed Test Methods to Improve Prediction of Adverse Health Effects

- Because these test methods are conducted using cells of human origin, the submitter contends that they will better reflect the human physiological response than current methods (i.e., *in vivo* rabbit pyrogen test, BET), and thus more effectively predict adverse effects.
- The submitter also contends that these test methods are capable of detecting both endotoxin and non-endotoxin pyrogens.



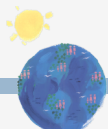
The Extent to Which the Test Methods Provide Advantages Compared to Current Methods

- Specific costs are not provided but the BRDs cite two factors (reagents, labor) in contributing to the cost of the proposed test methods.
- The proposed test methods are reportedly more labor-intensive than the *in vivo* rabbit pyrogen test or the BET; however, the proposed methods do appear to be adaptable to higher throughput, which could make them more cost effective.
- Based on the approximate time requirement for each assay (2 working days), these methods may take longer to perform than the rabbit pyrogen test or the BET, which can typically be completed in one day.



ICCVAM Conclusions on the *In Vitro* Pyrogenicity Test Methods

- These five *in vitro* test methods appear to have considerable potential for pyrogenicity testing.
- However, more information is needed from the sponsor prior to a formal review by an Expert Peer Review Panel.
- Pending receipt and review of the requested information, a determination will be made of the review priority for these test methods.



Possible Future Pyrogenicity Test Method Activities

- Dec 2005 Publish a *Federal Register* notice requesting
- public comment on the submission
 - all relevant data for consideration
 - candidates for a possible expert panel
- 18 Jan 06 PWG considers the test methods and recommends priority for future activity.
- 19 Jan 06 ICCVAM considers the PWG recommendations and public comments and recommends a priority for future activities.
- 2006 Possible ICCVAM Expert Panel review to:
- peer review the BRDs for the test methods
 - determine whether the data cited in the BRDs support draft ICCVAM test method recommendations regarding the proposed usefulness, limitations, and validation status of the test methods.

